

U.S. Appl. No. 09/476,485
Our Ref. No.: PHY-003U51/108236.119
Comm. Resp. to Examiner Inquiry dated November 22, 2004

EXHIBIT F

A copy of pages 20-21 of the instant application which provides support for determining the amino acid sequence identity between two proteins.

mutant thereof, wherein each FRIL family member molecule binds to a normally glycosylated FLT3 receptor has at least about 45% amino acid sequence identity with the amino acid sequence of another member of the FRIL family, preferably at least about 50% identity, even more preferably at least about 55% identity, still more preferably at least about 60% identity, and still more preferably at least about 65% identity with the sequence of the second protein. In the case of proteins having high sequence identity, the amino acid sequence of the first protein shares at least about 75% sequence identity, preferably at least about 85% identity, and more preferably at least about 95% identity, with the amino acid sequence of another member of the FRIL family.

Both amino acid sequence identity and nucleic acid sequence identity between two proteins or two nucleic acid molecules can be measured according to standard methods. For example, in order to compare a first amino acid sequence to a second amino acid sequence or a first nucleic acid sequence to a second nucleic acid sequence for the purpose of determining percentage identity between the two sequences, the sequences are aligned so as to maximize the number of identical amino acid or nucleic acid residues. The sequences of proteins sharing at least 50% amino acid sequence identity or the sequences of nucleic acids sharing at least 45% nucleic acid sequence identity can usually be aligned by visual inspection. If visual inspection is insufficient, the proteins or nucleic acids may be aligned in accordance with the FASTA method in accordance with Pearson and Lipman (*Proc. Natl. Acad. Sci. USA* 85:2444-2448, 1988), or, preferably, any of the methods described by George, D.G. et al., in *Macromolecular Sequencing and Synthesis, Selected Methods and Applications*, pages 127-149, Alan R. Liss, Inc. (1988), such as formula 4 at page 137 using a match score of 1, a mismatch score of 0, and a gap penalty of -1. From this method, percentage of sequence identity between the first and second amino acid sequences or between the first and second nucleic acid can be determined.

Other methods for determining amino acid or nucleic acid sequence identity are described in Feng and Doolittle (*Journal of Molecular Evolution* 25: 351-360, 1987) and Higgins and Sharp (*CABIOS* 5: 151-153, 1989).

Another method for determining amino acid or nucleic acid sequence identity between two proteins or nucleic acids is by using sequence analysis software with the default parameters specified therein. Various software packages exist including Sequence Analysis Software Package of the Genetics Computer Group (University of Wisconsin Biotechnology Center, Madison, WI), and the various BLAST programs of the National Center for Biotechnology (National Library of Medicine, Bethesda, MD).

Unless otherwise specified, percentage of amino acid sequence identity or percentage of nucleic acid sequence identity is determined using the basic BLAST program of the National Center for Biotechnology (National Library of Medicine, Bethesda, MD), using the default settings defined therein.

Another test for percentage identity of two nucleic acid sequences is whether they hybridize under normal hybridization conditions, preferably under stringent hybridization conditions. Thus, also included in the invention are proteins that are encoded by nucleic acid molecules that hybridize under high stringent conditions to a sequence complementary to SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, and/or SEQ ID NO: 7. The term "stringent conditions," as used herein, is equivalent to "high stringent conditions" and "high stringency." These terms are used interchangeably in the art.

Stringent conditions are defined in a number of ways. In one definition, stringent conditions are selected to be about 50°C lower than the thermal melting point (T_m) for a specific sequence at a defined ionic strength and pH. The T_m is the temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched sequence. Typical stringent conditions are those in which the salt concentration is at least about 0.02 M at pH 7 and the temperature is at least about 60°C. "Stringent conditions," in referring to percentage identity (e.g., homology) or substantial similarity in the hybridization context, can be combined